

*National Rural Development Programme 2014-2022*

*Measure 10.2 – Biodiversity*

*Project: TuBAvI-2 (2021-2024)*

**REPORT ON THE ACTIVITIES PERFORMED DURING THE THIRD YEAR**

**UNIPD**

The present report describes the activities performed from May 1<sup>st</sup>, 2023, to March 31<sup>st</sup>, 2024. The activities are described by Action.

**Action 1 – Phenotypic characterization of autochthonous breeds and species**

During the third year of the project, blood samples and phenotypic characteristics were collected on animals of the following species/breeds:

- *Collo Nudo Italiana* breed (species: *Gallus gallus*) (n = 48)
- *Oca Padovana* breed (species: *Anser anser*) (n = 53)

The collection of the above information led to the completion of the activity of recording morphometric measurements and collecting biological material (blood) required by the TuBAvI-2 project. Table 1 summarizes the qualitative phenotypic characteristics (shank and skin color) of all species/breeds involved in the project, and Figure 1 depicts the descriptive statistics (mean and standard deviation) of quantitative phenotypic characteristics (body weight, body length, shank length, shank circumference, wingspan, sternum circumference) recorded on the same species/breeds. Phenotypic characteristics are presented for males and females within breed and follow the guidelines of FAO (2012)<sup>2</sup>.

**Action 2 – Genetic characterization of Italian breeds and species**

The genetic analyses conducted by the external service/laboratory starting June 2023 enabled the acquisition of genotypes for all species/breeds involved in the project. Starting with DNA extracted from blood samples (about 2 mL) and stored in tubes with EDTA at -20°C, genotyping of animals was carried out using SNP molecular markers. Two distinct approaches were used, depending on the species:

- **Collo Nudo Italiana** and **Millefiori Piemontese breeds** (species: *Gallus gallus*) → Affymetrix Axiom Chicken 600K HD;
- **Anatra Mignon** and **Anatra Germanata Veneta** (species: *Anas platyrhynchos*), **Oca Padovana** (specie: *Anser anser*) and **Faraona Camosciata** (specie: *Numida meleagris*) breeds → low-coverage whole genome sequencing (WGS - coverage 4X).

The WGS approach for the species *Anas platyrhynchos*, *Anser anser* and *Numida meleagris* was used because there are no commercially available SNP chips with which to perform the same genotyping.

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<sup>2</sup> FAO. (2012). Phenotypic characterization of animal genetic resources. FAO Animal Production and Health Guidelines, (11).

### **Action 3 - Data and information consistency check**

The genotyping results were checked according to standard quality criteria. After applying filters to the genotypes, aimed at maximizing and assessing the accuracy of the results, the number of genotyped animals useful for subsequent analyses was as follows:

- *Collo Nudo Italiana* breed (species: *Gallus gallus*) (n = 47)
- *Millefiori Piemontese* breed (species: *Gallus gallus*) (n = 48)
- *Oca Padovana* breed (species: *Anser anser*) (n = 50)
- *Faraona Camosciata* breed (species: *Numida meleagris*) (n = 50)
- *Anatra Mignon* breed (species: *Anas platyrhynchos*) (n = 50)
- *Anatra Germanata* breed (species: *Anas platyrhynchos*) (n = 50)

### **Action 4 - Estimation of genetic and genomic indices and reproductive management in relation to new purposes**

The analyses following data quality control have been concluded for the species *Gallus gallus* and they are still in progress for the other species. The following genetic diversity indices were estimated for the chicken breeds: observed heterozygosity ( $H_o$ ), expected heterozygosity ( $H_e$ ), minimum allele frequency (MAF) and Runs of Homozygosity (ROH), i.e., portions of DNA that are identical on both chromosomes within the population and their respective islands of origin (Table 2).

### **Action 5 - Improvement of animal genetic resources of zootechnical interest, assessment of inbreeding and genetic diversity in the considered populations (from inbreeding calculation to data modification and collection in control station under controlled environment)**

In order to evaluate the enhancement of genetic resources and thus monitor the level of inbreeding in the *Gallus gallus* species, specific indices ( $F_{HOM}$  and  $F_{ROH}$ ) were estimated for each animal, based on the excess of homozygosity within the genome of the *Collo Nudo Italiana* and *Millefiori Piemontese* breeds. The results, in terms of within-breed averages, are shown in Table 2. The values, which can range from 0 to 1, indicate a low level of inbreeding of the two breeds and, therefore, an optimum level of genetic variability.

### **Action 6 - Monitoring of genetic diversity**

Data from the TuBAVI-2 project for the species *Gallus gallus* were merged with data of the same species from the previous project (TuBAVI). This allowed the estimation of the phylogenetic relationship by exploiting Reynolds genetic distances and paint a picture of the closeness between different breeds belonging to the Italian local poultry heritage (Figure 2).

### **Action 9 - Processing of the information collected (e.g., development of indicators and indices to minimize the environmental impact of breeding, calculation of planned mating, etc.)**

With the genotypes obtained from the TuBAVI-2 project and the previous TuBAVI project for the species *Gallus gallus*, it was possible to conduct a targeted investigation aimed at identifying genes involved in eggshell and shank color (Table 3).

## Action 10 – Information actions, dissemination and preparation of thematic technical reports and technical-scientific reports

The following activities were carried out under this action:

- a) participation in updating the project website (<https://www.pollitaliani.it/>);
- b) updating the data sheets of Veneto poultry species/breeds:
  - Species: *Meleagris gallopavo*. Breeds: Bronzato Comune, Ermellinato di Rovigo.
  - Species: *Gallus gallus*. Breeds: Millefiori di Lonigo, Ermellinata di Rovigo, Pepoi, Padovana (Dorata, Camosciata and Argentata), Polverara (Bianca and Nera), Robusta Maculata, Robusta Lionata;
- c) participation in national and international congresses with oral/poster presentation:
  - Cendron, F., Cassandro, M., Penasa, M. (2023). Copy number variants in 23 Italian local chicken breeds. In: Book of Abstracts of the 25th National Congress of the Animal Science and Production Association (ASPA), 13-16 June, Monopoli (BA), Italy. ITALIAN JOURNAL OF ANIMAL SCIENCE, 22(Suppl. 1):99-100. (Abstr. O481). (Oral Presentation).
  - Cendron, F., Penasa, M., Cassandro, M. (2023). Genome-wide detection and analysis of copy number variation in Italian indigenous chicken breeds. In: Proceedings of the 12th European Symposium on Poultry Genetics, 8-10 November, Hannover, Germany, p. 46. (Poster).
- d) publication of the following scientific papers:
  - Cendron, F., Cassandro, M., Penasa, M. (2024). Genome-wide investigation to assess copy number variants in the Italian local chicken population. JOURNAL OF ANIMAL SCIENCE AND BIOTECHNOLOGY, 15:2.
  - Perini, F., Cendron, F., Lasagna, E., Cassandro, M., Penasa, M. (2024). Genomic Insights into Shank and Eggshell Color in Italian Local Chickens. POULTRY SCIENCE, 103:103677.

## FIGURES AND TABLES

**Table 1 – Shank and skin color of sampled animals.**

Breed	Species	Sex	N° animals	Shank color	Skin color
Collo Nudo Italiana	<i>Gallus gallus</i>	Males	21	Yellow	White
		Females	26	Yellow	White
Faraona Camosciata	<i>Numida meleagris</i>	Males	25	Dark orange	Grey
		Females	26	Brown	Grey
Anatra Mignon	<i>Anas platyrhynchos</i>	Males	12	Orange	White
		Females	43	Orange	White
Anatra Germanata Veneta	<i>Anas platyrhynchos</i>	Males	14	Orange	White
		Females	41	Orange	White
Oca Padovana	<i>Anser anser</i>	Males	23	Orange	White/Pink
		Females	39	Orange	White/Pink

**Table 2 – Indices of genetic diversity and coefficients of inbreeding.**

Breed	N° animals	MAF		He		Ho		F <sub>HOM</sub>		F <sub>ROH</sub>	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Collo Nudo Italiana	47	0.31	0.17	0.67	0.12	0.65	0.14	0.00	0.07	0.00	0.00
Millefiori Piemontese	48	0.25	0.20	0.57	0.12	0.60	0.12	0.15	0.41	0.02	0.01

MAF = minimum allele frequency; He = expected heterozygosity; Ho = observed heterozygosity; F<sub>HOM</sub> = coefficient of inbreeding based on homozygosity regions; F<sub>ROH</sub> = coefficient of inbreeding estimated based on runs of homozygosity. SD = standard deviation.

**Table 3 – Genes responsible for eggshell and shank color.**

Shank		Eggshell	
Chromosome and position	Genes	Chromosome and position	Genes
1:100867039-101831504	<i>CHODL, TMPRSS15, NCAM2</i>	2:61184687-61271239	<i>JARID2</i>
2:38800153-38800475	<i>EOMES, CMC1, AZI2, RBMS3</i>	4:26751167-30211214	<i>PCDH18</i>
Z:11112490-12254700	<i>SLC1A3, RANBP3L, SLC45A2</i>	5:15963249-15987149	<i>PNPLA2, SLC25A22</i>
Z: 18925613-18969905	<i>ERCC8</i>	12:15839118-16014277	<i>MITF, FAM19A4, ARL6IP5, UBA3</i>
Z: 23446708-23450910	<i>F2RL1</i>	Z :10028822-10307280	<i>NPR3, TARS, ADAMTS12, SLC45A2</i>
Z:31376546-32699330	<i>NFIB, ZDHHC21, CER1, PSIP1, BNC2, TYRP1</i>		
Z:78846780-79172113	<i>CDKN2A, CDKN2B</i>		
Z:78846780-79172113	<i>MTAP, FEM1C</i>		
Z:78846780-79213873	<i>TRIM36, GRAMD3</i>		

Figure 1 – Mean and standard deviation of quantitative phenotypic characteristics by breed and sex of the animals.

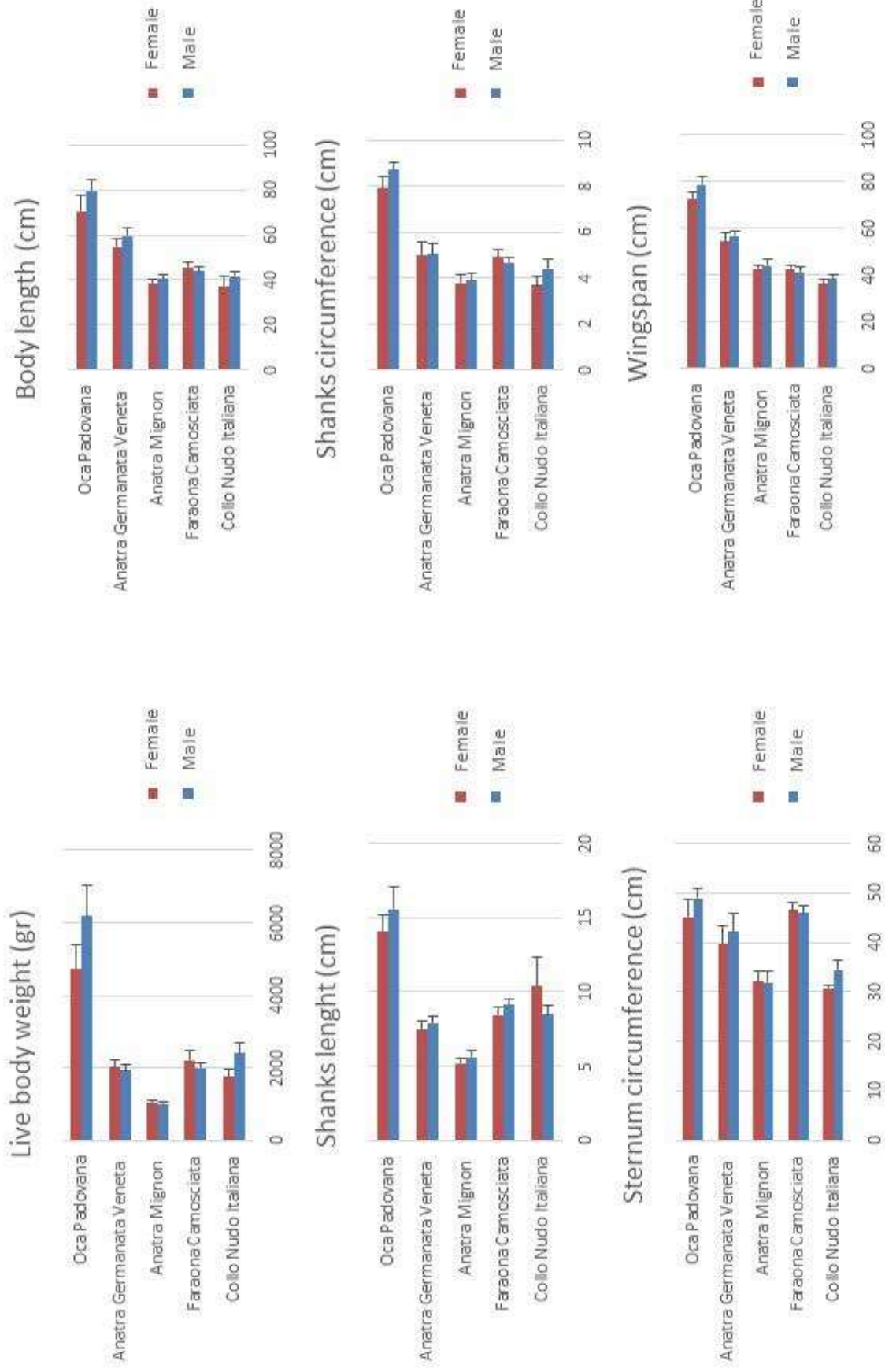
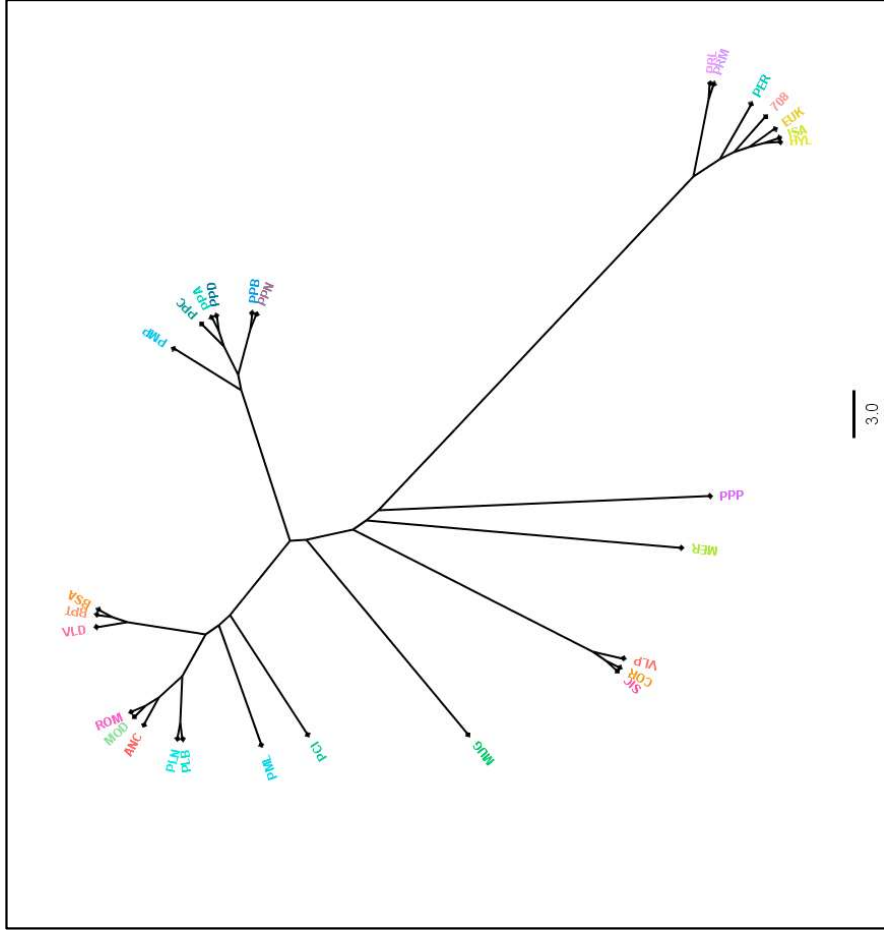


Figure 2 – Phylogenetic tree for chicken breeds (species: *Gallus gallus*) from the TuBAVI and TuBAVI-2 projects, obtained using Reynolds genetic distances.



Ancona (ANC), Bianca di Saluzzo (BSA), Bionda Piemontese (BPT), Cornuta di Caltanissetta (COR), Collo Nudo Italiana (PCI), Livorno Bianca (PLB), Livorno Nera (PLN), Millefiori Piemontese (PMP), Mericanel della Brianza (MER), Modenese (MOD), Mugellese (MUG), Ermellinata di Rovigo (PER), Millefiori di Lonigo (PML), Padovana Argentata (PPA), Polverara Bianca (PPB), Padovana Camosciata (PPC), Padovana Dorata (PPD), Polverara Nera (PPN), Pepoi (PPP), Robusta Lionata (PRL), Robusta Maculata (PRM), Romagnola (ROM), Siciliana (SIC), Valdarnese (VLD), Valpatani (VLP), Broiler Ross 708 (708), Eureka (EUK), Hy-lyne (HYL), ISA Brown (ISA)